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**Immunologische Studien über den
Einfluß von isologen Lymphozyten
auf das Wachstum von
EHRlich-Aszites-Tumor**

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Immunological Studies of the Influence of Isologous Lymphocytes on the Growth of the Ehrlich-Ascites-Tumor*

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In recent years it became known that lymphocytes play an important role in the tuberculin- and the homotransplantation-reaction and a series of autoimmune diseases (2, 3, 4, 10, 12, 14, 15, 17, 18, 19, 21, 23, 26). Furthermore it could be demonstrated that lymphocytes of immunized animals are prone to suppress the growth of various transplanted tumors in contrast to lymphocytes of non immunized animals (8, 11, 13, 22, 27). Cells of lymphnodes and spleens of sensitized animals destroy tumor cells *in vivo* as well as *in vitro*; (1, 5, 6, 7, 9, 20). The objective of this experiment was to demonstrate the influence of active immunization on the intra-peritoneal growth of EHRlich-ascites-tumor cells**. The effect of mononuclear cells of sensitized animals on the growth of EHRlich-ascites-tumor following passive intravenous or intraperitoneal transfer of lymphocytes has been investigated.

Methods

(I) Active Immunization with Non-Viable EHRlich-Ascites-Tumor

Inbred mice of the C57/Bl strain of the same age and female sex were used as test animals. Body weight of these animals was between 17—19 g at the beginning of the experiment. The EHRlich-ascites-tumor was obtained by sterile puncture of the peritoneal cavity 10 days after inoculation and was homogenized in a glass homogenizer of POTTER-ELVEHJEM at +2 °C for 10 min. Thereafter one part of FREUND's adjuvant and three parts of homogenized ascites were emulsified. 40 mice received 0.2 ml of this

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emulsion intramuscularly. 7 days later an intraperitoneal injection of viable EHRlich-ascites tumor cells in a dose of 0.1 ml was given to 20 mice and 0.2 ml to the other 20 of these animals. Control animals of the same age, weight and sex received without pretreatment 0.1 or 0.2 ml of EHRlich-ascites-tumor cells intraperitoneally. In order to determine the effect of active sensitization concerning tumor growth the following examinations were performed in all animals (20):

- (1) daily weights;
- (2) determination of survival of the animals after transfer of the EHRlich-ascites-tumor cells;
- (3) determination of the number of mitoses. Starting at the 10th day after beginning of the experiment ascites from 5 animals was aspirated under sterile conditions with 3 day intervals and the smears stained according to FEULGEN. The incidence of mitoses was stated after counting of 2000 cells.

(II) Passive Immunization by Intravenous Injection of Lymphocytes from Sensitized Animals

Homogenized ascites was mixed in a ratio of 3:1 with FREUND's adjuvant. Femal mice from the inbred strain C57/Bl with a body weight between 17 and 19 g were sensitized intramuscularly with three injections each to 0.1 ml with 3 day intervals. 8 days after the last injection the animals were sacrificed; spleen, thymus and lymph-nodes were removed and the organs shredded under sterile conditions. The cell suspension in HANK's solution was centrifuged for 10 min at 1200 rpm and the number of cells counted in a FUCHS-ROSENTHAL chamber. The survival rate of these cells was 90% according to the nigrosin test.

45 mice of the inbred strain C57/Bl of a body weight between 17 and 19 g received 0.1 ml EHRlich-ascites-tumor cells intraperitoneally. On the following day the cell suspension of the sensitized animals in a dose of 30×10^6 cells was given into the tail vein. To prevent any anaphylactoid reaction the total dose was divided into several portions. Control animals were mice of the inbred strain C57/Bl of the same weight, sex and age. These animals received lymphocytes of untreated animals in a dose of 30×10^6 cells intravenously instead of lymphocytes of sensitized animals, after 0.1 ml of EHRlich-ascites-tumor cells had been given intraperitoneally on the preceding day.

To observe the growth of the tumor under passive immunization the following criteria were set:

- (1) daily weights;
- (2) determination of survival time after transfer of the EHRlich-ascites-tumor cells;
- (3) rate of mitosis.

(III) Passive Intraperitoneal Injection of Lymphocytes from Sensitized Animals

In an additional experiment lymphocytes of sensitized inbred mice of the strain C57/Bl were given to mice of the same strain intraperitoneally. The dose of 25×10^6 cells was administered on four consecutive days. Simultaneously controls received instead of lymphocytes from sensitized animals lymphocytes from untreated animals in the same dose.

The following investigations were performed:

- (1) daily weights;
- (2) determination of survival time after transfer to the tumor cells;
- (3) rate of mitosis.

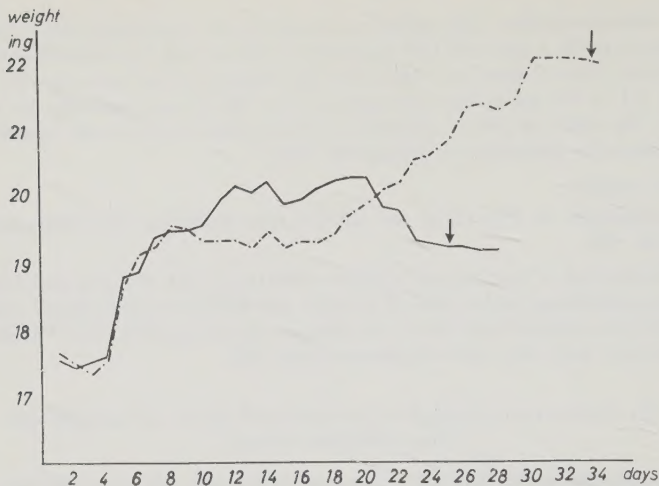


Fig. 1. Weight curves and 50% mortality ($\downarrow$) of 20 sensitized and 20 untreated mice after intraperitoneal injection of 0.1 ml EHRlich-ascites-tumor. --- Mice sensitized with 0.2 ml homogenized EHRlich-ascites-tumor together with FREUND's adjuvant. — Untreated animals.

Results

(I) Results of Active Sensitization

Weight curves and the 50% mortality rate of C57/Bl mice receiving 0.1 ml or 0.2 ml of EHRlich-ascites-tumor cells intraperitoneally following active sensitization and those of the controls are presented graphically in Fig. 1 and 2.

As it can be seen in these graphic representations the sensitized and the control animals showed a parallel increase in body-weight during the first three weeks. In control animals receiving 0.1 ml EHRlich-ascites-tumor the weight increase was higher between the 10th and 20th day than in the presensitized mice; this difference, however, was statistically not significant. In the presensitized mice a rapid weight gain after the 20th day was observed, when control animals had succumbed to tumor cachexia. The survival time was shorter in control animals by 5 to 7 days as compared with the presensitized animals. No mice survived in either serie.

The results of the determination of mitosis rate are listed in Table I.

The frequency of mitosis observed was lower on the average in sensitized animals than in the controls. The highest rate of mitosis was seen after day 10 and 15 after transfer of EHRlich-ascites-



Fig. 2. Weight curves and 50% mortality ($\downarrow$) of 20 sensitized and 20 untreated mice after intraperitoneal injection of 0.2 ml EHRlich-ascites-tumor. - - - - Mice sensitized with 0.2 ml homogenized Ehrlich-ascites-tumor together with FREUND's adjuvant. ——— Untreated animals.

Table I

Determination of the mitosis rate after counting of 2000 EHRlich-ascites-tumor cells in actively sensitized mice and controls.

Day after inoculation of Ehrlich-ascites-tumor	Rate of mitosis			
	in sensitized mice; ascites i.p.:		in untreated mice; ascites i.p.:	
	0.1 ml	0.2 ml	0.1 ml	0.2 ml
10	0.9%	1.3%	1.9%	1.5%
15	1.2%	1.0%	1.9%	1.6%
20	0.8%	0.7%	1.2%	1.7%
25	1.0%	0.2%	0.1%	0.3%

tumor decreasing to a minimum until death. Several hours prior to death the ascites cells revealed almost no mitosis.

(II) Results Following Intravenous Transfer of Lymphocytes from Sensitized Animals

Weight curves and 50% mortality of C57/Bl mice receiving 30×10^6 lymphocytes from sensitized or in the controls from untreated animals after intraperitoneal injection of EHRlich-ascites-tumor cells are shown in the following Fig. 3.

The weight curves are similar until the 10th day. No initial weight gain was observed here in contrast to the actively sensitized

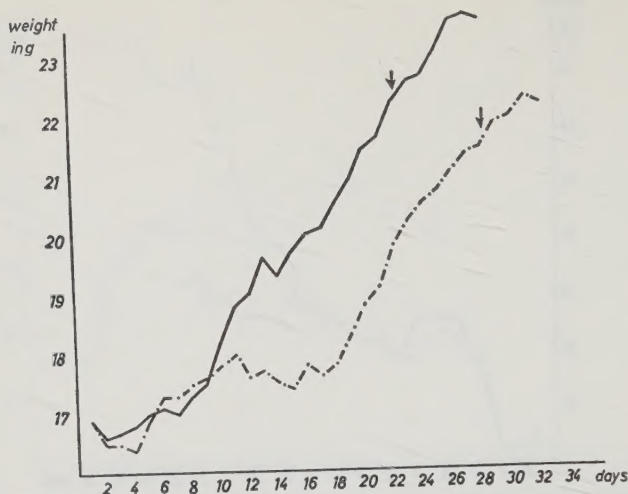


Fig. 3. Weight curves and 50% mortality (↓) of 45 mice which received lymphocytes of sensitized or 35 untreated mice intravenously after intraperitoneal injection of 0.1 ml EHRlich-ascites-tumor. - - - Mice receiving lymphocytes of sensitized animals. — Mice receiving lymphocytes of untreated animals.

Table II

Determination of the mitosis rate after counting of 2000 EHRlich-ascites-tumor cells after intravenous transfer of lymphocytes of sensitized or untreated mice. 0.1 ml EHRlich-ascites-tumor was injected intraperitoneally.

Day after inoculation of Ehrlich-ascites-tumor	Rate of mitosis	
	Mice injected i.v. with lymphocytes of sensitized mice; ascites i.p. 0.1 ml	Mice injected i.v. with lymphocytes of untreated mice; ascites i.p. 0.1 ml
10	1.2%	2.0%
14	1.4%	2.3%
17	1.0%	2.1%
20	0.3%	1.6%
23	0.1%	0.6%

animals. Beyond the 10th day a steady weight gain was present in the controls whereas a similar weight increase in the sensitized animals was delayed until day 18. The 50% mortality of the controls was reached on the 23rd day after injection of ascites; the same mortality in the animals receiving lymphocytes from sensitized mice had occurred on day 28.

The results of the determination of mitosis rate are stated in Table II.

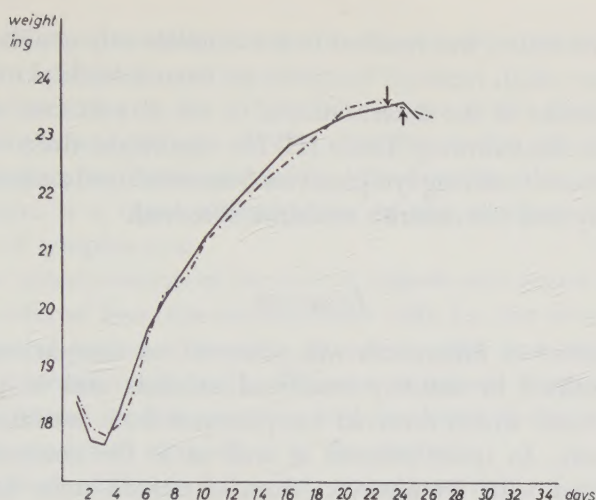


Fig. 4. Weight curves and 50% mortality ($\downarrow$) of 25 mice which received lymphocytes from sensitized or 25 untreated mice intraperitoneally after intraperitoneal injection of 0.1 ml EHRLICH-ascites-tumor. ---- Mice with lymphocytes from sensitized mice. — Mice with lymphocytes from untreated mice.

Table III

Determination of the mitosis rate after counting of 2000 EHRLICH-ascites-tumor cells in mice which had received lymphocytes from sensitized and untreated mice intraperitoneally. 0.1 ml ascites was injected intraperitoneally.

Day after inoculation of Ehrlich-ascites-tumor	Rate of mitosis	
	Mice injected i.p. with lymphocytes of sensitized mice; ascites i.p.: 0.1 ml	Mice injected i.p. with lymphocytes of untreated mice; ascites i.p.: 0.1 ml
10	1.9%	2.0%
14	1.7%	1.9%
18	0.6%	0.8%
22	0.2%	0.05%

The percentage of mitosis in sensitized animals was smaller as in the controls. The rate of mitosis decreased during the observation time in the same manner as described in the first group.

(III) Results of Intraperitoneal Transfer of Lymphocytes from Sensitized Animals

As can be seen in the graphic representation (Fig. 4) the weight curve from mice which received lymphocytes from sensitized animals intraperitoneally did not deviate in any way from the controls.

The 50% mortality was reached in the controls only one day before that of mice which received lymphocytes from sensitized mice.

The results of the determination of the mitosis rate are summarized in the following Table III. No significant differences between the mice receiving lymphocytes from sensitized animals intraperitoneally and the controls could be observed.

Discussion

A number of differences was observed on comparison of the results obtained in actively sensitized animals and in passively sensitized mice which received lymphocytes from sensitized mice intravenously. In these animals as well as in the controls which were injected with lymphocytes from untreated mice no initial weight gain was observed which can be seen usually after transfer of EHRLICH-ascites-tumor in untreated animals. We believe that this represents an effect of the intravenous injection of lymphocytes. Beginning at day 10 after inoculation of EHRLICH-ascites-tumor cells control animals receiving lymphocytes from untreated mice show an almost linear weight increase until the time of the 50% mortality is reached. Animals, however, treated with lymphocytes from sensitized mice had no weight gain until day 18; consecutively, they revealed a similar weight curve. It must be assumed that the injection of lymphocytes from sensitized animals has caused the delay in weight gain. At the time of the 50% mortality the difference in weight was about 1 g (5% of the total body weight). The most striking criteria in the two groups of actively sensitized animals was the rapid weight gain starting after day 18 which was absent in the controls. A dependency existed between the dose of EHRLICH-ascites-tumor administered and the weight gain observed: after injection of 0.2 ml EHRLICH-ascites-tumor the weight gain was larger than after administration of 0.1 ml and 50% mortality counted from the day of ascites transfer occurred earlier.

Except of a brief initial weight loss nearly identical weight curves were seen in mice which had received lymphocytes from sensitized and untreated mice intraperitoneally and in untreated animals. 50% mortality in the actively sensitized animals and those which had received lymphocytes from sensitized animals intravenously was reached about 7 days later than in the control groups. 50% mortality in animals which received lymphocytes from sensitiz-

ed mice intraperitoneally, occurred only one day after the controls. No mice survived in any group.

Contradictory to the other observations is the identity of the weight curves and the time of 50% mortality in the groups receiving the lymphocytes intraperitoneally. Presumably the most likely explanation is a rapid disintegration of the intraperitoneally administered lymphocytes.

The determination of the rate of mitosis was begun on day 10 after transfer of EHRlich-ascites-tumor cells, i.e. the time when the formation of ascites allowed a puncture, and was continued at regular intervals until death. The rate of mitosis began to decline from the first to the last puncture and was lower in the actively and passively sensitized animals as in the controls.

Survival of hosts following transplantation of a tumor is dependent upon the properties of the tumor on the one hand and the immunologic defense mechanisms of the host on the other. The EHRlich-ascites-tumor is a rather rapidly growing tumor whose growth could not be suppressed to a significant degree by means of presensitization. Active sensitization leads to a prolongation of survival of hosts as compared to controls by as many as 6 to 7 days. The prolongation of the survival of the animals, which received lymphocytes from sensitized mice intravenously was similar to the actively sensitized group, implying that this finding is due to the influence of these lymphocytes.

The rapid weight increase in the actively sensitized mice after the 18th day which was not present in controls is not clearly understood. It can only be suspected that this finding can be ascribed to circulating antibodies which accelerate the growth of the tumor, a phenomenon, described in the literature as enhancement (16, 25). Corresponding experiments utilizing serum from actively sensitized animals for transfer were not carried out because of the difficulty in obtaining an adequate quantity of isologous immune serum.

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Summary

After transfer of lymphocytes from actively sensitized animals the growth of EHRlich-ascites-tumor cells is delayed as could be demonstrated by weight curves, 50% mortality and mitosis rate of the tumor cells in the host. The weight curves of the actively sensitiz-

ed animals revealed a rapid weight increase after the 18th day which was not observed in the non sensitized control animals. The 50 percent mortality was reached later and the mitosis rate was diminished. The possibility of an enhancement of the transferred tumor cells by circulating antibodies is discussed. The tumor growth was not impaired after intraperitoneal transfer of lymphocytes from sensitized animals.

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Lebenslauf

Am 23. Dezember 1930 wurde ich als Sohn des Beamten Willy Schmidt und seiner Ehefrau Emma, geb. Kämmer, in Halle-Saale geboren. Besuch der Volks- und Oberrealschule. Reifeprüfung 1953. Anschließend 2 Semester Medizin an der Humboldt-Universität Berlin. Im Jahre 1955 Immatrikulation an der Freien Universität Berlin, nachdem ich zuvor die Reifeprüfung beim Senator für Volksbildung in Berlin bestanden hatte. Nach Unterbrechung des Studiums Immatrikulation an der Friedrich-Alexander-Universität Erlangen-Nürnberg. Die ärztliche Vorprüfung bestand ich im Jahre 1964, das medizinische Staatsexamen im Februar 1968.

